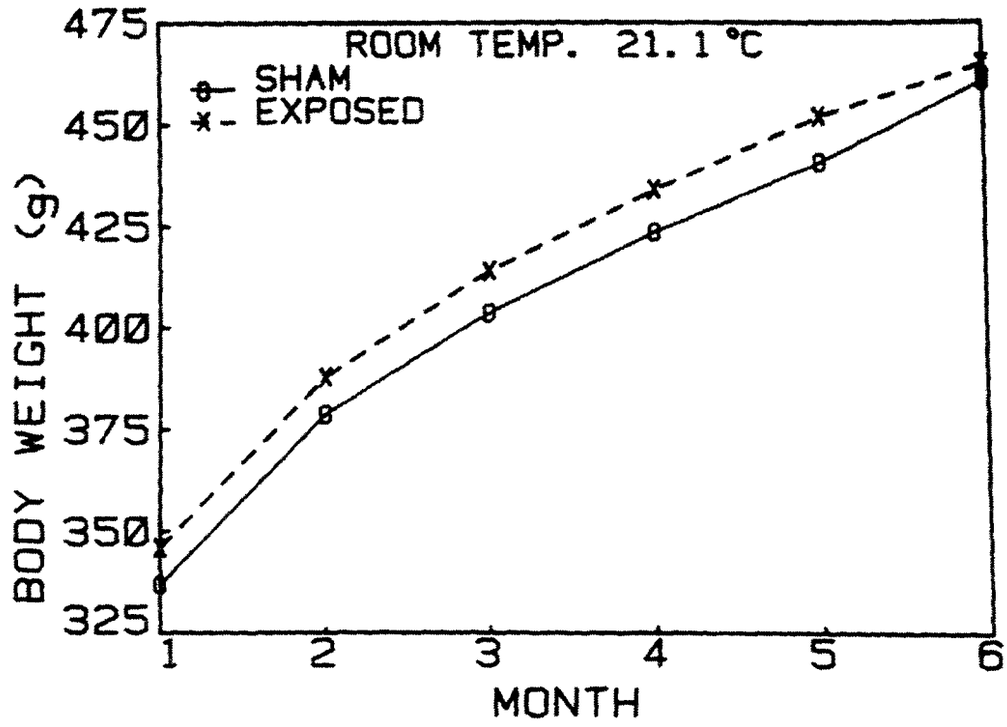

Figure 1. Mean body mass of the 6-mo exposure.

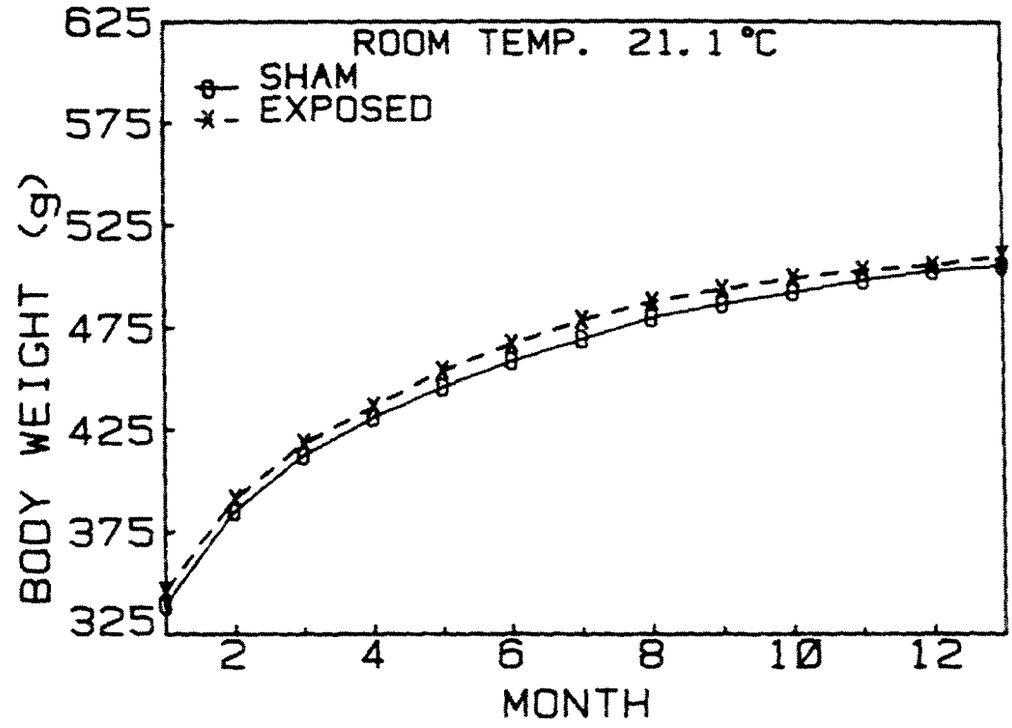

Figure 2. Mean body mass of the 12-mo exposure.

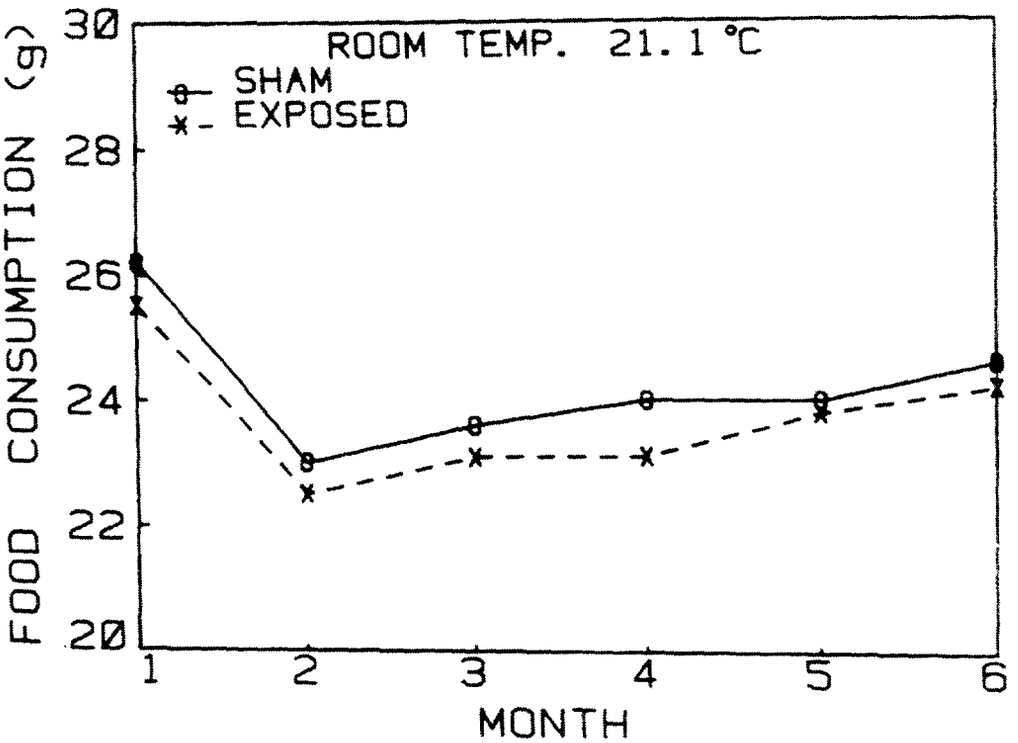

Figure 3. Mean food consumption of the 6-mo exposure.

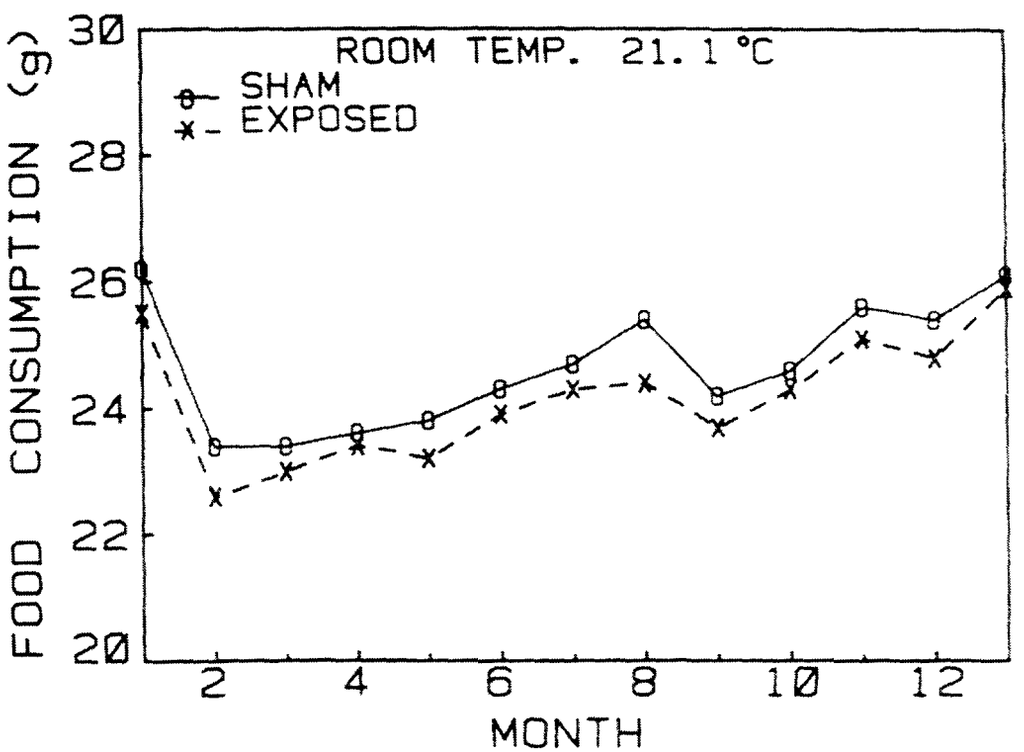

Figure 4. Mean food consumption of the 12-mo exposure.

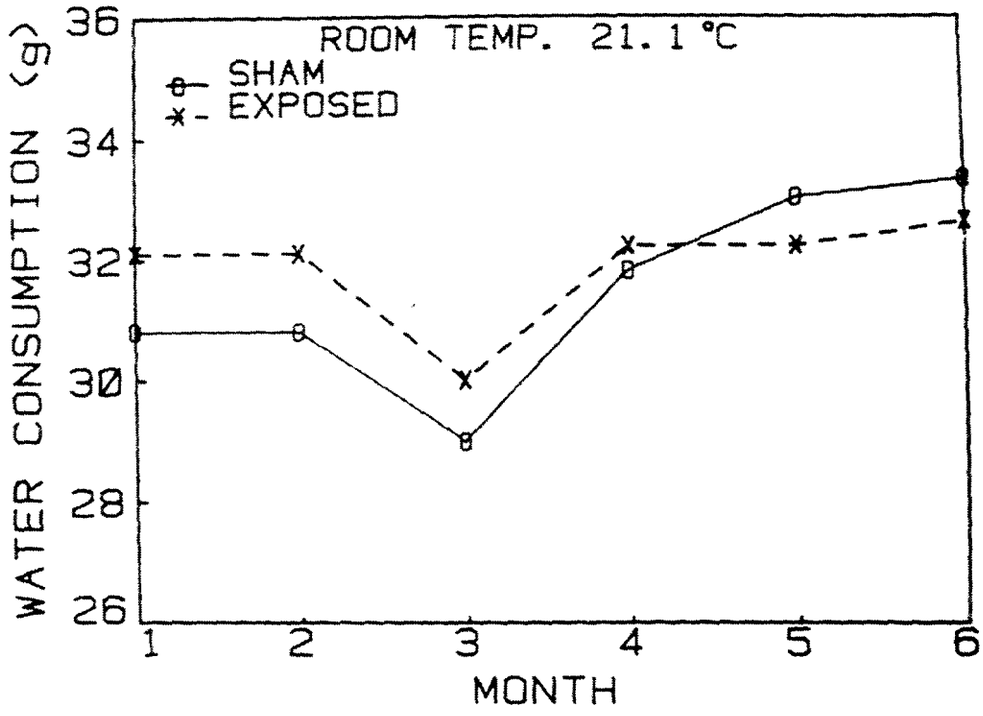

Figure 5. Mean water consumption of the 6-mo exposure.

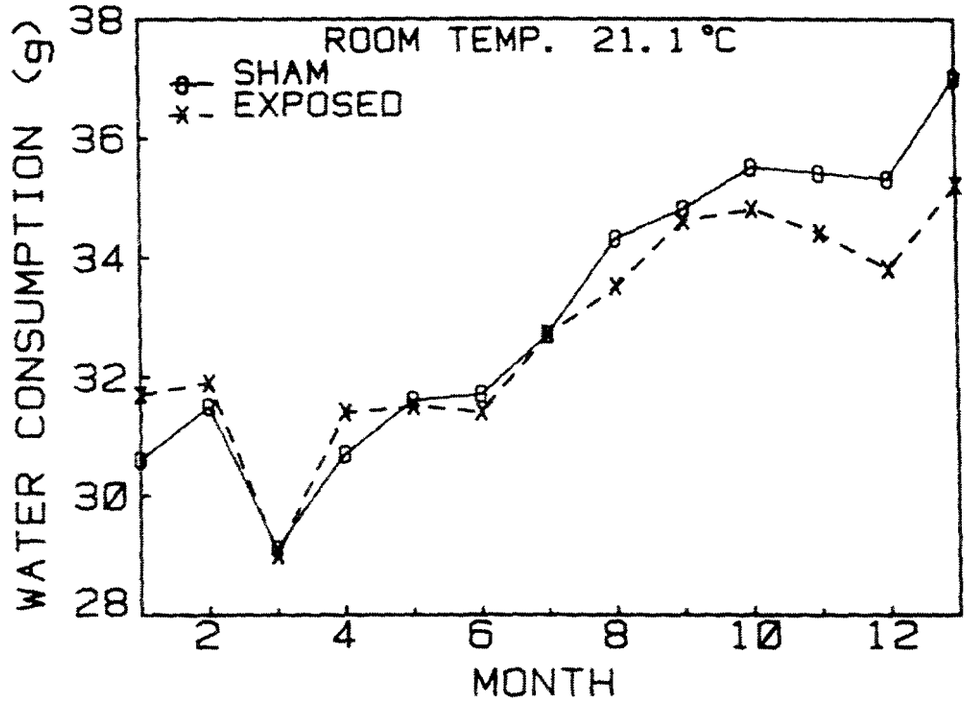

Figure 6. Mean water consumption of the 12-mo exposure.

Immune Competence Evaluation

Since the mid-1970s an increasing number of studies concerning the immunological response of various experimental animals to microwave irradiation have appeared in the scientific literature. This material provides a basic framework for inquiry concerning microwave irradiation but, when viewed as a whole, reveals inconsistencies and inadequacies that stimulate controversy about the significance of the basic biological findings and their role as a potential human-health hazard.

Increased lymphoid blastogenesis occurred after mitogen stimulation in mice exposed to 25-kHz electric fields (Bollinger et al., 1974) and in monkeys exposed to 27-MHz fields (Prince et al., 1972). Czerski (1974) reported increased numbers of peripheral lymphoblasts in exposed rats and mice; Huang et al. (1977, 1980) made the same observation. Studies by Liburdy (1979, 1980) indicate that both radiofrequency radiation (RFR) and steroid treatment affect lymphocyte circulation in mice. Liburdy speculates that the steroid release associated with thermal stress and the process of thermoregulation are significant factors responsible for RFR effects on the immune system. The studies of Smialowicz (1979) and Roszkowski et al. (1980) indicate that microwave-hyperthermia-evoked changes in hemopoiesis and lymphopoiesis can for the most part be attributed to nonspecific stress responses or to the direct thermal effect on immunocompetent cells. When studied in vitro, the metabolism of lymphocytes and reticuloendothelial cells is stimulated by hyperthermia at 38-40^{o}C and is dramatically inhibited, then irreversibly damaged, upon exceeding 43^{o}C (Roszkowski et al., 1979). Therefore, changes in lymphoid blastogenic reactivity to mitogens and synthesis of antibodies by lymphocytes in these in vitro studies seem to be influenced by microwave energy deposition. This may be the basis of the stimulatory effect of microwaves on the maturation of B-lymphocytes, leading to the reported increased presence of complement-receptor (CR^{+}) cells (Schlagel et al., 1980), increased mitogen response, and increased plaque-forming ability (antibody production) (Wiktor-Jedrzejczak et al., 1980; Sulek et al., 1980; Liddle et al., 1980).

The stimulatory effects observed in the cited studies have not been reproduced by others and exhibit discrepancies which may be explained by variations in level and duration of exposure, especially the studies performed at low-field intensities (Smialowicz, 1979, 1981; Huang, 1980; McRee et al., 1980; Hellstrom et al., 1981).

The stage of maturity of an animal's immune system may be a factor in determining susceptibility to modification by microwave irradiation. When immunological assessments were made at 3 and 6 wk of age, no consistent differences in plaque-forming ability, mitogen response, or natural killer (NK) cell activity were observed between irradiated and sham-irradiated mice exposed in utero to 2450-MHz microwaves at an incident power density of 28 mW/cm^2 for 100 min daily from day 6 to day 18 of pregnancy (Smialowicz et al., 1982b). These results contrast with alterations seen by Wiktor-Jedrezejak et al. (1977) after postnatal exposure of mice: A mild stimulatory effect on splenic lymphocytes was indicated by increased responsiveness to mitogens and increased antibody-forming spleen cells. In rats an enhanced lymphocyte responsiveness was found when they were exposed in utero at 425 MHz (Smialowicz et al., 1982a) and when irradiated in utero and during early postnatal life (Smialowicz, 1979; Smialowicz et al., 1982b). These studies suggest that only postnatal irradiation of mice may induce changes in lymphocyte responsiveness, whereas rats show increased lymphocyte responsiveness when exposed either in utero or postnatally. This variation between rats and mice may be related to species differences in maturation of the immune system.

Smialowicz et al. (1982a) suggest that exposure to microwave radiation tends to modify immunological competence in rats, resulting in an SAR in excess of their basal metabolic rate. Bowhill (1981) affirms that exposure to acute microwave irradiation or an exposure lasting from one to several days stimulates immune system activity. Bowhill further speculates that there is a biphasic reaction to the immune system after exposure. That supposition is not documented by experimental data, however, and is contrary to findings in long-term exposure experiments with mice (Bollinger et al., 1974), monkeys (Prince et al., 1972), and rats (Kunz et al., 1983).

During the most recent rat experiment conducted at the University of Washington, several immunological tests were performed on 10 exposed and 10 sham-exposed rats after a 1-yr exposure. The most significant finding was the enhanced splenic lymphocyte response to various mitogens and the absolute increase in splenic B and T cells of the exposed rats. That long-term study provided for a broad screening of immunological competence, including the evaluation of splenic lymphocytes for response to various B- and T-cell-specific mitogens as an in vitro measure of lymphoid cell functionality, DNA synthesis, and cell division. These included the plant lectins Con A and phytohemaglutinin (PHA) that specifically stimulate T-cell populations. The B-cell-specific mitogens bacterial lipopolysaccharide (LPS) of E. coli, PPD, and PWM were also used. Enumerations of B and T cell populations in the spleen were determined by using direct and indirect immunofluorescent techniques respectively.

Splenocyte populations were also assayed for frequency of complement-receptor positive cells. Spleen cells obtained from 10 exposed and 10 sham-exposed rats were assayed in duplicate for ability to form plaque-forming cells in response to sheep red-blood cells (SRBC), as a measurement of antibody-producing ability. One-half of each group was injected with SRBC, and the other half with phosphate-buffered saline (PBS) as a control. No significant differences were seen between the exposed and sham-exposed rats in the percentages of CRP cells in the spleens. In the animals immunized with SRBC there was a slight (statistically insignificant) increase in plaque per spleen for the exposed animals relative to the sham-exposed, indicating no major impairment of the antigen presentation system and no deficiency of the B-cells' ability to produce antibodies in the presence of functional T cells.

Relative to the sham-exposed group, the exposed animals had a significant increase in both splenic B and T cells. This is suggestive of a general stimulation of lymphopoiesis or of selective lymphoid-cell sequestration in the animals exposed to RFR. The mitogen stimulation studies revealed a significant difference in the patterns of response to various B- and T-cell-specific mitogens. The data indicate, using the multivariate

Hotelling T^2 statistic, that the exposed animals had a nonsignificant increase for PHA and a significant increase for LPS and PWM at the .05 level. The exposed animals showed a significantly increased response for Con A and a decreased response for PPD at the .01 level. The results of the mitogen test may indicate a selective effect on the lymphoid system by RFR, enabling the spleen cells to have a pronounced and selective mitogen response.

Our objectives were to extend the observations of the previous study and to use more sensitive methodologies with larger numbers of animals in order to obtain more reliable results. Specifically, we investigated the proliferative response in vitro of thymus cells, splenocytes, and bone marrow cells to a panel of polyclonal activators. Using flow cytometric techniques in combination with specific antibodies to T- and B-lymphocyte subpopulations, we determined the cellular composition of the thymus gland, spleen, and bone marrow. Finally, using the colony-forming assay in agar (CFU-C), we quantified hematopoietic progenitor cells of the monocyte/macrophage and granulocyte lineages in the bone marrow compartment.

Methods

Study Design and Method of Analysis. Eighty rats were randomly divided into two groups of 40 each. One group received microwave radiation; the other served as an unexposed control group. After the 6-mo exposure period, on each of 5 kill days eight rats were sacrificed and their spleen, thymus, and bone marrow cells assayed according to the following plan:

Kill Day	No. Exposed	No. Controls
1	5	3
2	6	2
3	2	6
4	4	4
5	3	5

After the 12-mo study, 40 rats were sacrificed and their organs removed and assayed on 4 kill days as follows:

Kill Day	No. Exposed	No. Controls
1	5	5
2	5	5
3	5	5
4	5	5

To accommodate the imbalance present in the two groups with respect to the random effect of "day," we used the General Mixed Model Analysis of Variance Program BMDP-3V to analyze these data (Table 1). The method of estimation was "maximum likelihood"; computations were done on a DEC-10 computer.

Exposure Facility and Dosimetry. The exposure facility consisted of 50 waveguides for active exposure and 50 for sham exposure. The active waveguides were powered by two 2450-MHz pulse-microwave generators, each capable of delivering a maximum of 10-W average power at 800 pps with a 10-μsec pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of .48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power-meter-output analysis indicated that these exposure conditions resulted in average SARs ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat. Details of the exposure facility and dosimetry have been reported before (Guy et al., 1983a; Chou et al., 1984).

TABLE 1. STATISTICAL MODEL

$$Y_{ijk} = u + \underset{\text{group (fixed)}}{\alpha_i} + \underset{\text{day (random)}}{b_j} + \underset{\text{error (random)}}{e_{ijk}} \qquad \begin{matrix} i = 1,2 \\ j = 1...5 \end{matrix}$$

assumptions: $b_j \sim N(0, \sigma_b^2)$

$e_{ijk} \sim N(0, \sigma_e^2)$

Test statistic for the null hypothesis of no group effect is the likelihood-ratio test of $H_0: \alpha_i = 0$; p-value is obtained by referencing the asymptotic distribution of this statistic, which is $\chi^2(1)$.

Mitogen Stimulation Studies. At the end of the 6- and 12-mo exposures, the animals were removed from the waveguide at approximately 1.5-min intervals, taken to the adjoining necropsy room, anesthetized, and exsanguinated. The spleen, thymus, and femur were removed aseptically, and an approximate 8% section of spleen and thymus was removed for histopathological analysis. The remaining portion of each spleen and thymus was delivered to the immunology laboratory for analysis within 15 min of removal.

Cell Preparations.

1. Single-cell suspensions of spleen and thymus were prepared by gentle teasing from the capsule and suspension in RPMI 1640 medium.
2. Bone-marrow cells were flushed from femoral cavities with cold medium. A single-cell suspension was made by repeated aspiration in a pipette. Erythrocyte lysis was accompanied by treatment of the cell suspension with 0.83% buffered ammonium chloride.

Colony-Forming Unit-Culture. Our laboratory used a modified method of Bradley and Metcalf (1969) to detect CFU-C. The cell suspension of marrow was mixed with 0.3% Bacto-agar in MEM-alpha medium containing 25% heat-inactivated pooled human serum. Other culture additives included glutamine, antibiotics, 1% bovine serum albumin, 2-ME, and 10% PWM spleen-conditioned medium. The conditioned medium served as a source of colony-stimulating activity (CSA). The cells were added to give a final concentration of 1×10^5 cells per ml of agar, and 1 ml was placed in each 35- x 10-mm petri dish to gel (Falcon Plastics, Oxnard, CA). After the gel stage, the petri dishes were incubated in a CO_2 incubator at 37°C with 5% CO_2 to 95% air. After 7 days, the numbers of colonies (clusters of over 50

cells) were counted using an inverted microscope. The means and standard deviation of triplicate plates were calculated for each experimental condition.

Cytochemical Examination of Cells Grown in Agar Gel. All colonies in an agar gel can be stained cytochemically to detect the presence of chloracyl esterase. Granulocyte and macrophage clusters of colonies can be easily distinguished from one another by the production of reaction products from naphthol AS-D chloroacetate. After the colonies had been counted, the agar medium was transferred from the petri dishes to glass slides. To absorb the liquid, Whatman #1 filter paper was placed on the surface. This process produced a flattened, dried preparation, ready for fixation in 10% cold neutral buffered formalin for 10 min. Fixed preparations were then washed in water, dried, and incubated for 45 min at 37^{o}C in freshly prepared substrate. The slides were washed in water, air-dried, and counterstained with hematoxylin. We examined the slides under a low-power microscope to count total number of colonies and the number with all cells containing red-reaction production (neutrophil), the number without product (macrophage), and the number in which some cells contain reaction product (mixed neutrophil-macrophage). The data were expressed as the fraction of total colonies that are macrophage, neutrophil-macrophage, and neutrophil. The number of CFU-C that belong to each of the categories could also be calculated. From these data, shifts in the frequencies and number of progenitors, granulocyte, granulocyte-macrophage, and macrophage in the tissue could be detected. Table 2 details the variables analyzed.

TABLE 2. CFU-C AND MITOGEN STIMULATION VARIABLES ANALYZED IN STUDY

A. CFU-C total and differential counts

1. Total colonies
2. Macrophage colony count
3. Granulocyte colony count
4. Mixed colony count

B. Mitogen stimulation assay

Differences in observed scale of ^{125}IUdR cpm
(stimulated response - control;
e.g., Con A - medium control)

To compare stimulation indices in previous study, we also analyzed differences in natural log scale
[ln(stimulated-response/control);
e.g., ln(Con A/medium control)]

1. For thymus

Con A
PHA
PWM
medium control

2. For spleen

LPS
PHA
PPD
Con A
PWM

Separate likelihood-ratio tests were performed on each variable to test H_0: α_i=0 (at 0.05 level).

Measurement of DNA Synthesis. Aliquots of 0.1 ml of cell suspensions placed into a 96-well flat-bottomed microtiter plate (Linbro-Flow Labs, Inglewood, CA) were cultured and the DNA synthesis activity measured. After the appropriate culture period, cells were incubated with 5.0 μCi/ml of ^{125}I-iododeoxyuridine (^{125}IUdR) (2000 Ci/mmol, specific activity; New England Nuclear) and 10^{-6}M 5-fluorodeoxyuridine (Aldrich Chemical Co., Milwaukee, WI) for 4 h and then harvested by an automated cell harvester. The radiolabel incorporation was determined by a gamma counter. The data were expressed as net counts per minute (experimental cpm - control cpm) and as a stimulation index (E/C). Table 2, Section B, shows the variables analyzed.

B- and T-Cell Enumeration. Spleen cell, bone marrow, and thymocyte suspensions were prepared for each animal, as previously described for the mitogen assays. Then, 2×10^6 cells of each tissue were pelleted in 1-ml microfuge tubes and stained for the detection of either B or T cells as follows:

B cells -- 50 μl of fluorescein isothiocyanate (FITC) $(Fab')_2$ goat antirat Ig (polyvalent) antibody was added to the cell suspensions and incubated on ice for 30 min

T cells -- a proper volume of FITC monoclonal mouse anti-Thy 1.1 antibody was added to cell suspensions and incubated on ice for 30 min

The cells were then suspended in media, centrifuged to remove the excess FITC antibody, and resuspended in media containing 0.1% sodium azide. Samples were either analyzed in an orthocytofluorograph or fixed with 2% paraformaldehyde for analysis at a later time. Table 3 lists the variables analyzed.

TABLE 3. B- AND T-CELL VARIABLES ANALYZED IN STUDY

Quantitation of B and T cells by expression of surface immunoglobulin and Thy 1.1 antigens, respectively, and mean population intensity of each antigen per cell basis:

1. Thymocytes and splenocytes
    a. Percentage of all viable cells expressing cell-surface antigens
    b. Percentage of low light scatter expressing cell-surface antigens
    c. Percentage of high light scatter expressing cell-surface antigens
2. Bone marrow cells
    a. Percentage of all viable cells expressing cell-surface antigens
    b. Percentage of low light scatter expressing cell-surface antigens
    c. Percentage of high light scatter expressing cell-surface antigens
    d. Percentage of medium light scatter expressing cell-surface antigens

Quantitation of cell populations in each tissue using correlated forward-angle light scatter (cell size) and right-angle light scatter (cellular complexity):

1. Thymocytes and splenocytes
    a. Total cells per organ x 10^{-6}
    b. Percentage of total cells in low-light-scatter population
    c. Percentage of total cells in high-light-scatter population
2. Bone marrow cells
    a. Total cells per organ x 10^{-6}
    b. Percentage of total cells in low-light-scatter population
    c. Percentage of total cells in high-light-scatter population
    d. Percentage of total cells in medium-light-scatter population

Separate likelihood-ratio tests were performed on each variable to test H_0: $\alpha_i=0$ (at 0.05 level).

Orthocytofluorograph Analysis. By quantitative fluorescence the various cell populations were measured with an Ortho System 50H Cell Sorter and Ortho model 2150 computer and analysis unit (Ortho Diagnostic Systems, Westwood, MA). The instrument is equipped with dual argon and krypton ion high-power lasers for optimum two-color [e.g., fluorescein isothiocyanate (FITC) and rhodamine isothiocyanate (RITC) fluorescence] analysis. The computer unit allows complex gating analysis of any combination of up to four available parameters; for example, forward- and right-angle scatter and green and red fluorescence. Data are stored on 5-Mbyte hard disks allowing later display and reanalysis. As illustrated in Figure 7, correlated forward-angle light scatter (cell size, y axis) and right-angle light scatter (complexity of cell structure, x axis) was used to select cell populations from each tissue to be assayed for B- and T-cell specific markers respectively.

For example, in Figure 7 the rectangle (region 1) encompasses all intact cells in each tissue. Regions 2 and 3 are low- and high-light-scatter populations respectively. In the case of bone marrow (BM), region 4 includes cells with medium forward scatter and high right-angle-scatter (e.g., granulocytes), hereafter referred to as the "medium-light-scatter population."

Figure 8 depicts the fluorescence histograms of splenocyte and thymocyte populations. In panels A and B, aliquots of splenocytes were exposed to FITC-goat antidog C3 (negative control) and to FITC-goat antirat IgG respectively. Regions (channels 80-200) were determined where 5% of the nonspecifically stained cells (panel A) were included in the B-cell population (panel B). Panels D and C illustrate the T-cell stain, FITC Thy 1.1, on thymocyte suspensions and its control respectively.

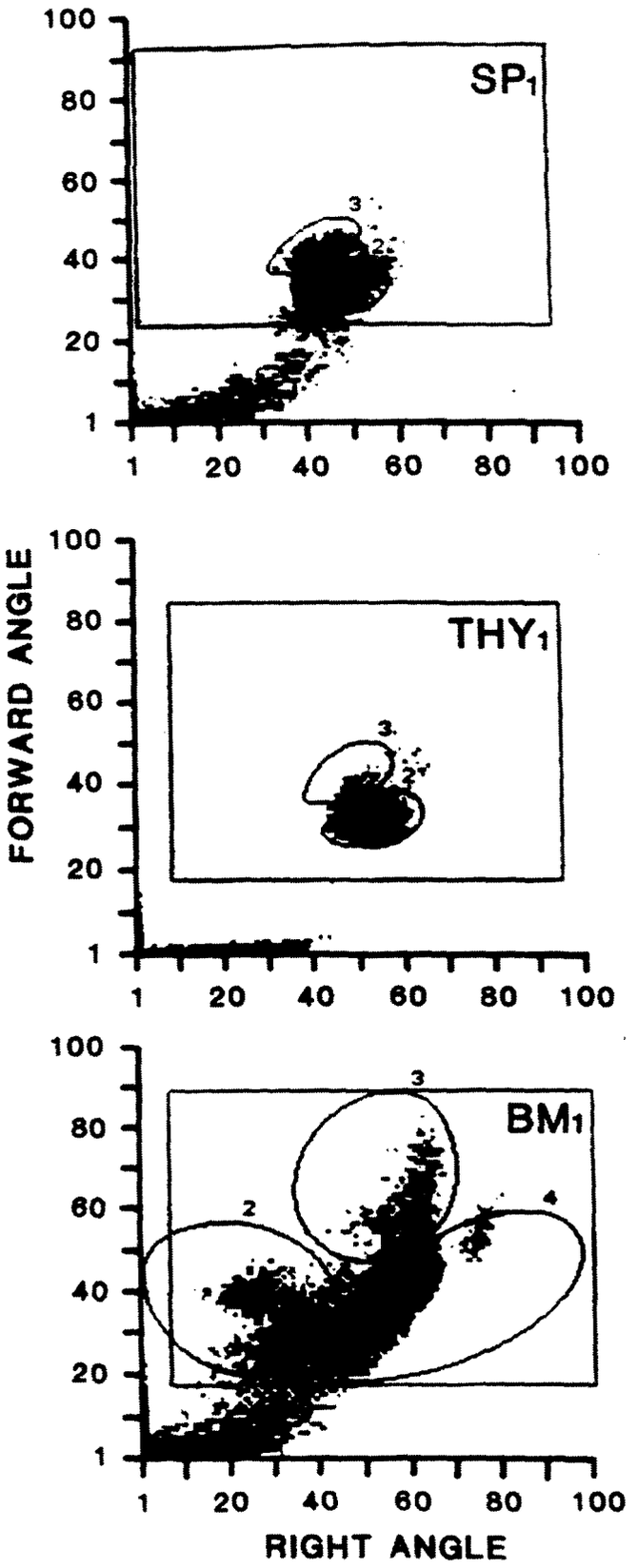

Figure 7. Scatter histograms from the orthocytofluorograph 50H.

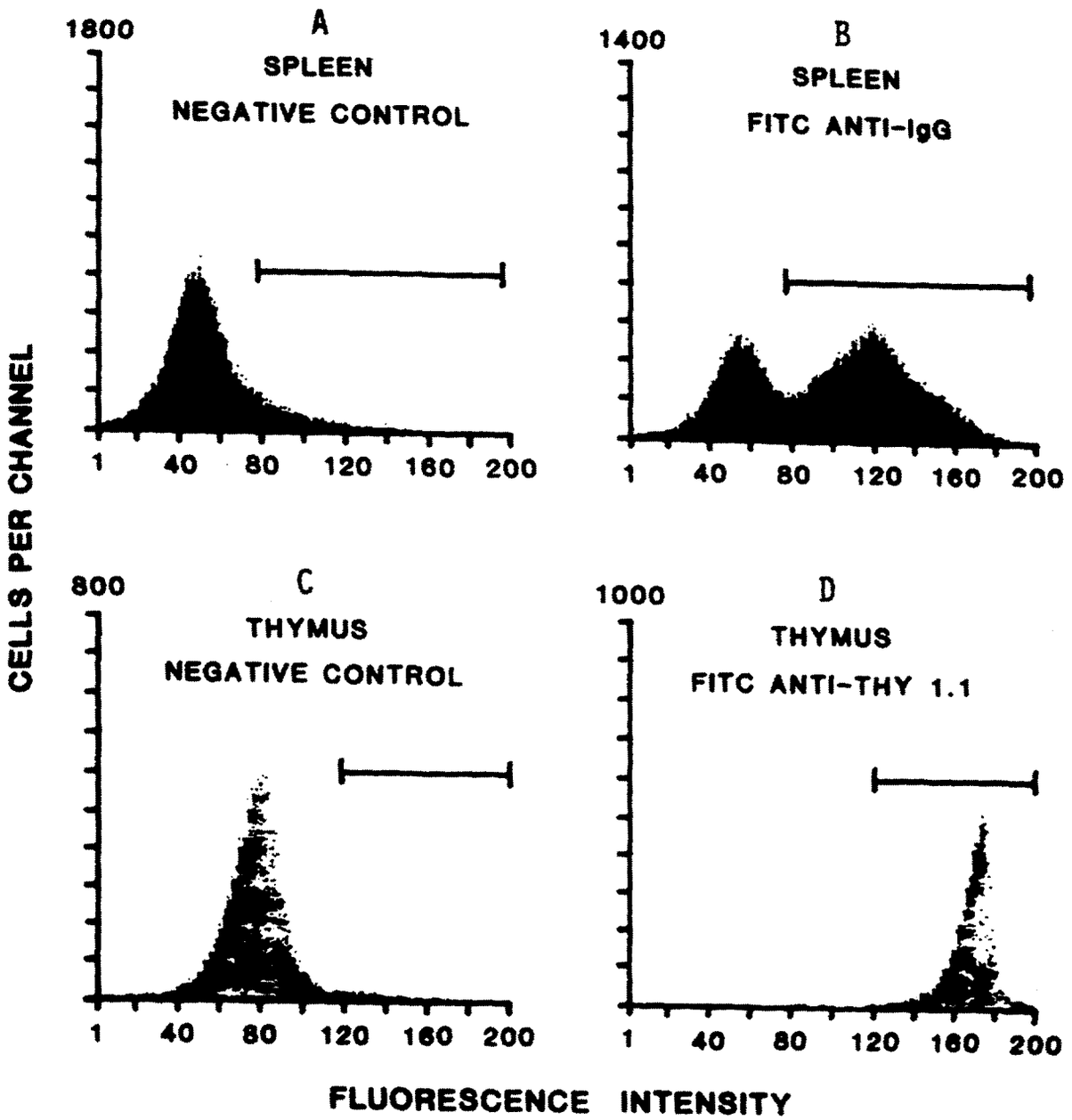

Figure 8. Fluoresence histograms of splenocyte and thymocyte populations.

Results

CFU-C Assay. The numbers of hematopoietic progenitor cells were quantitated in the femurs of exposed and sham-exposed rats, using the method of Bradley and Metcalf (1969). As shown in Figure 9, the data demonstrated that rats exposed for 6 mo had significantly elevated numbers of total ($p = 0.014$), macrophage ($p = 0.046$), and granulocyte colonies ($p = 0.016$); no differences were observed in the numbers of mixed colonies. As depicted in Figure 10, similar results were observed after exposure for 12 mo: exposed rats had significantly elevated numbers of total ($p < 0.001$), macrophage ($p < 0.001$), and granulocyte colonies ($p < 0.079$). After both 6- and 12-mo exposures, the cellularity of the femoral cavities was not different between experimental and control animals.

Mitogen Responses. To detect functional alterations induced by RFR irradiation in subpopulations of lymphoid cells from the spleen and thymus, we cultured single-cell suspensions of each organ in vitro with the optimal concentration of mitogens and assayed for DNA synthesis on the peak day of DNA synthesis response (preliminary data, not shown). All DNA synthesis responses were analyzed both in the observed scale and the natural logarithmic (ln) scale. Differences in the observed scale (stimulated - control) reflect excess stimulated response over baseline in counts per minute. Differences in the ln scale [ln(stimulated) - ln(control) = ln(stimulated/control)] are ln-transformed stimulation indices; the ln transformation decreases the influence of large outliers. No significant differences between exposed and sham-exposed rats were observed in the proliferation of thymocytes to Con A, PHA, and PWM (Table 4) after 6- and 12-mo of exposures. This result was unchanged when rats with outlying values were excluded from the analysis.

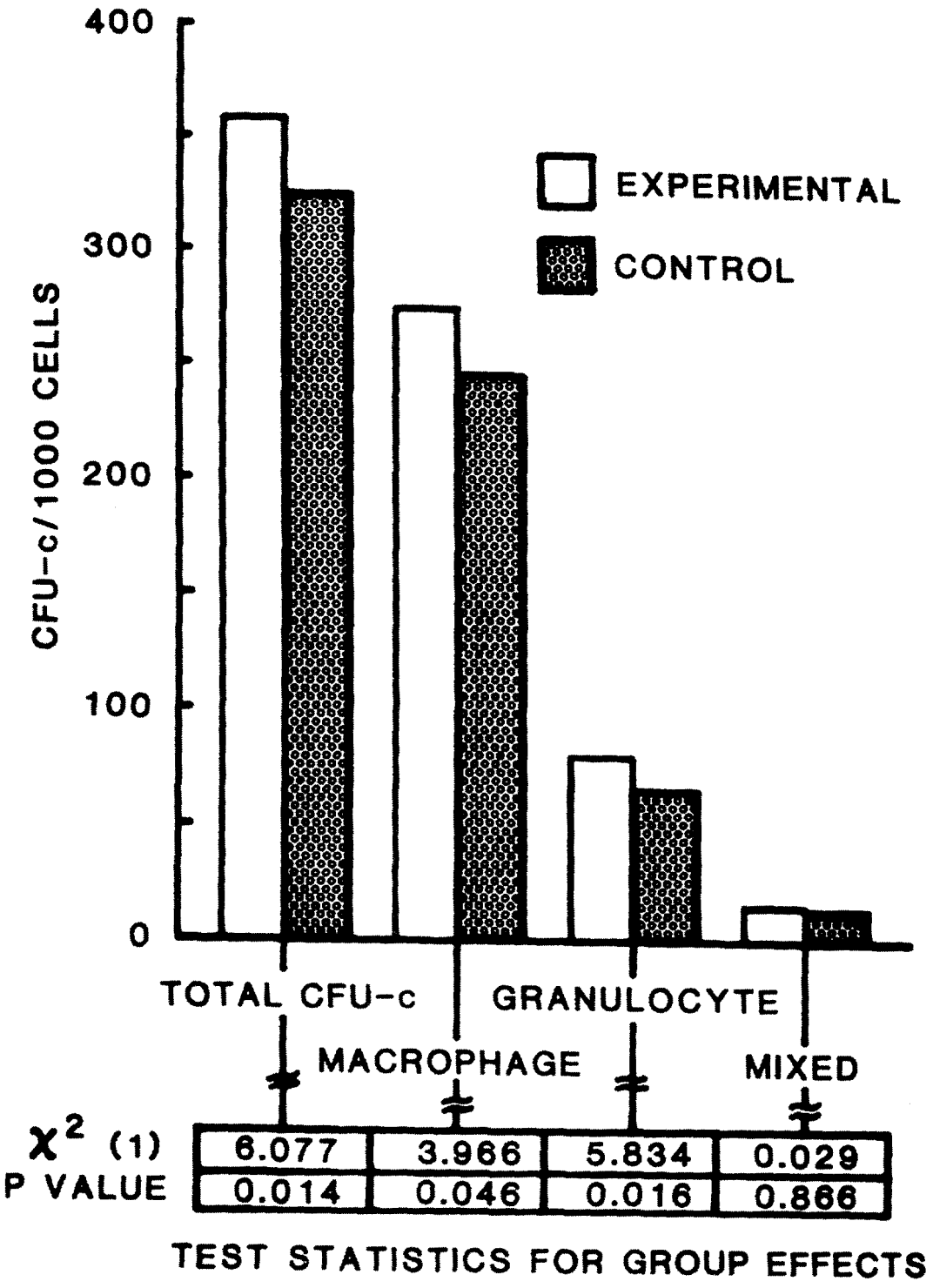

Figure 9. Effects of 6-mo exposure on the levels of total, macrophage, granulocyte, and mixed CFU colonies.

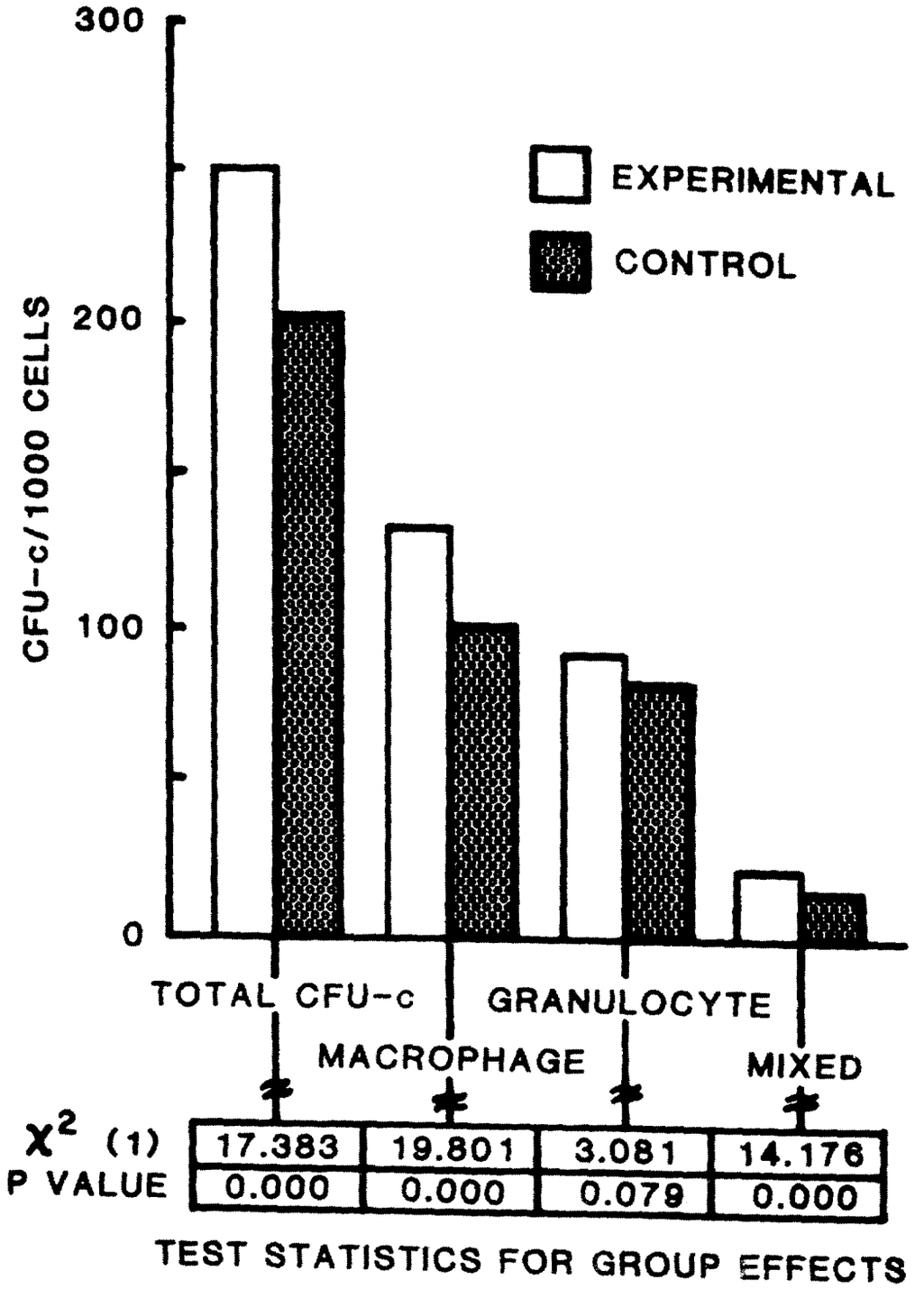

Figure 10. Effects of 12-mo exposure on the levels of total, macrophage, granulocyte, and mixed CFU colonies.

Test statistics for group differences with respect to DNA synthesis response of splenocytes to a panel of mitogens are given in Table 5. No significant group differences were observed for the mitogens at the .05 level. The LPS and PPD results were somewhat sensitive to data anomalies mentioned in the discussion. Analysis of the DNA synthesis response of splenocytes after 12 mo of exposure revealed no significant differences between experimental and control animals.

TABLE 4. ANALYSIS OF PROLIFERATIVE RESPONSE OF THYMOCYTES IN VITRO TO PHA, PWM, AND CON A

Exposure period (mo)	Culture stimulant	Mean ^{125}IUdR cpm (standard error)		Chi-squared (1) (p value)
		Exposed	Unexposed	Observed scale
6	Con A[a]	43,332 (7,176)	33,957 (4,918)	3.051 (0.081)
6	PHA[a]	3,507 (2,055)	809 (89)	1.771 (0.183)
6	PWM[a]	584 (248)	224 (43)	2.131 (0.144)
12	Con A[a]	91,122 (10,927)	87,913 (14,748)	0.068 (0.794)
12	PHA[b]	2,055 (371)	2,166 (674)	0.023 (0.879)
12	PWM[c]	1,492 (251)	1,778 (398)	0.389 (0.533)

a: n = 40
b: n = 29
c: n = 38

TABLE 5. ANALYSIS OF RESPONSES OF SPLENOCYTES IN VITRO FROM IRRADIATED AND CONTROL ANIMALS TO LPS, PHA, PPD, CON A, AND PWM

Exposure period (mo)	Culture stimulant	Mean ^{125}IUdR cpm (standard error) Exposed	Unexposed	Chi-squared (1) (p value) Observed scale
6	LPS[a]	16,610 (3,252)	18,039 (3,174)	2.813 (0.094)
6	PHA[a]	29,913 (6,258)	28,997 (4,441)	0 (0.983)
6	PPD[a]	2,592 (570)	2,446 (421)	1.264 (0.264)
6	Con A[a]	41,545 (7,199)	50,730 (6,689)	0.046) (0.830)
6	PWM[a]	21,815 (4,300)	26,093 (3,766)	1.337 (0.248)
12	LPS[b]	16,504 (2,572)	12,428 (2,688)	2.490 (0.115)
12	PHA[c]	8,678 (2,230)	10,141 (4,414)	0.122 (0.727)
12	PPD[d]	184 (86)	206 (72)	0.042 (0.838)
12	Con A[a]	129,723 (21,570)	110,942 (20,896)	1.075 (0.300)
12	PWM[a]	4,528 (1,092)	4,571 (1,804)	0.0 (0.983)

a: n = 40
b: n = 39
c: n = 38
d: n = 30

Quantitation of T and B Cells by Flow Cytometric Techniques. Thymocyte suspensions prepared from exposed and sham-exposed rats for 6- and 12-mo groups were each subsequently stained with FITC goat antirat IgG (B cells) and FITC anti-Thy 1.1 (T cells). The cells were analyzed on the orthocyto-fluorograph for six parameters (Table 6). No significant differences between groups were observed in the percentage of B (s-Ig) or T (Thy 1.1) cells in the thymii. The population mean expression of s-Ig and Thy 1.1 cell-surface antigen did not differ significantly between exposed and sham-exposed animals. Due to technical difficulties with the immuno-chemical staining on the fifth day of the experiment, three experimental and five control animals were excluded from the analysis of the 6-mo study. Animals exposed for 12 mo demonstrated marginally significant reduction in the percentage of B cells in the entire thymus gland and, as expected, reduction in the fraction of cells with low light scatter that carry s-Ig. In addition, the mean expression of Thy 1.1 antigen was reduced ($p = 0.029$) in cells whose light scatter is consistent with that of small lymphocytes.

As depicted in Table 7, analysis of marrow and spleen cells from exposed and sham-exposed rats for the presence of B and T cells revealed the following: (a) no evidence of group differences in the percentage of B and T cells and in the mean expression of s-Ig and Thy 1.1 (or B or T cells); (b) marginally significant group differences ($p = 0.05$) on the percentage of total viable B cells in the marrow, where the exposed animals had fewer B cells in the marrow; (c) no other statistically significant group differences. After 12 mo of RFR exposure, the T-cell population in the marrow was highly significant, whereas the mean surface density of s-Ig was significantly reduced in both the low- and high-light-scatter spleen-cell population. No other group differences were observed (see Table 8).

In Table 9, the analysis of correlated light-scatter parameters revealed that exposed animals had significantly fewer marrow cells with low-light-scatter characteristics than did sham-exposed rats. No group differences with respect to the other light-scatter characteristics were evident for either 6 or 12 mo of RFR exposure.

TABLE 6. ANALYSIS OF CELLULAR COMPOSITION IN THYMUS GLAND OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value)			
	6 Months		12 Months	
Variable analyzed	B cells	T cells	B cells	T cells
Percentage of entire population bearing antigen	2.042 (0.153)	3.007 (0.083)	3.740 (0.053)	0.738 (0.390)
Percentage of low light scatter bearing antigen	0.061 (0.805)	1.680 (0.195)	3.996 (0.046)	0.851 (0.356)
Percentage of high light scatter bearing antigen	0.351 (0.533)	0.550 (0.458)	ND	ND
Mean expression of antigen over entire population	0.309 (0.578)	1.781 (0.182)	0.024 (0.877)	3.358 (0.067)
Mean expression of antigen in low-light-scatter population	0.159 (0.691)	0.066 (0.797)	0.198 (0.656)	4.761 (0.029)
Mean expression of antigen in high-light-scatter population	0.258 (0.611)	1.961 (0.161)	ND	ND

TABLE 7. ANALYSIS OF CELLULAR COMPOSITION IN BONE MARROW AND SPLEEN OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value) 6 Months			
	B Cells		T Cells	
Variable analyzed	Marrow	Spleen	Marrow	Splee
Percentage of entire population bearing antigen	3.831 (0.050)	0.945 (0.331)	0.900 (0.343)	1.229 (0.268
Percentage of low light scatter bearing antigen	1.972 (0.160)	0.730 (0.393)	0.307 (0.579)	0.982 (0.322
Percentage of medium light scatter bearing antigen	3.551 (0.060)	ND	0.969 (0.325)	ND
Percentage of high light scatter bearing antigen	1.468 (0.226)	0.823 (0.364)	2.443 (0.118)	0.739 (0.390
Mean expression of antigen over entire population	0.189 (0.664)	0.012 (0.913)	0.043 (0.835)	1.537 (0.215
Mean expression of antigen in low-light-scatter population	0.006 (0.938)	0.852 (0.356)	0.191 (0.662)	0.094 (0.759)
Mean expression of antigen in medium-light-scatter population	0.530 (0.467)	ND	0.758 (0.384)	ND
Mean expression of antigen in high-light-scatter population	2.257 (0.133)	0.000 (0.996)	0.655 (0.418)	1.925 (0.165)